C·R·E·S®

CENTER FOR REPRODUCTION OF ENDANGERED SPECIES

REPORT

SUMMER 1997

DNA Is Where You Find It

Male condors appear identical to female condors in the eyes of human observers. One of the first and most basic characteristics that orients the behavior of an individual to others of its species is gender. For many species of higher organisms, sexual identity—maleness or femaleness—orients and limits behavior.

When the California condor wild population became almost extinct in the early 1980s, chromosomally based sex determination offered a rigorous and definitive method for condor gender identification. However, no one had ever done it. CRES scientists were invited to take up the challenge: to determine the sex of California condors at a time when all condors were wild hatched, and only a single bird was held in captivity. Using blood samples obtained from Andean condors of known sex held in zoos, cytogenetics specialist Arlene Kumamoto developed the necessary procedures, and we all hoped they would work on the California condors. Condors probably survive today, at least in part, because her techniques did work and the wild California condors had their sex determined.

In the early 1980s, with the California condor critically endangered, CRES scientists took up the challenge and discovered an accurate method of sex determination in birds. This is Sisquoc at two weeks of age at the San Diego Wild Animal Park in 1983.

Each time wild condors of unknown sex were handled to fit radio tags and collect blood samples for health evaluation, chromosomal studies were undertaken. After the celebrated hatching of California condor chicks in captivity, their health evaluation included sex identification by chromosomal analysis when they had grown enough to have blood taken safely. However, this technique, while decisive and accurate, was also time consuming and invasive. At this time, there was strong interest in a noninvasive method of identifying sex. It is possible to sex birds using endocrinological methods on fecal samples, an identification based on relative proportions of female and male steroid hormones. However, this method needed samples from sexually mature birds in breeding condition, a circumstance that would set back plans for the raising and pairing of captive California condor chicks.

> ***…it is becoming clear that DNA may be present in various types of samples that were, up to now, considered unlikely sources of genetic information.***

Portions of DNA from females would be expected to be different from that of males. This possibility was explored through a collaborative study between scientists at Texas Tech University, Los Alamos National Laboratory, and CRES. They were able to create a method for DNA-based sex identification

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Lesser Antilles Iguana
Working to save an endangered lizard.
See page 3.

Dr. David Western Accepts Award
1997 Conservation Medalist honored.
See page 4.

CRES Endowment for the Future
How your gift can help CRES.
See back cover.

DNA Is Where You Find It

Continued from page 1

for California condors that became the new standard technology. This method required far less blood, could be conducted more quickly, and was useful for analyzing a larger number of birds at one time, when compared to the old method of directly visualizing the chromosomes. After initially working out the technique, CRES scientist Leona Chemnick found that she could identify the sex of California condor chicks using only the membrane and blood remaining in the eggshell of a hatchling. It was no longer necessary to take blood samples from condor chicks to determine their sex.

Most recently, a UCSD graduate student, Anita McElroy, has successfully demonstrated that another method, involving DNA amplification (called PCR, or polymerase chain reaction), can be used to detect female-specific DNA sequences in California condors. Such a method would be even more rapid and useful when handling a large number of samples than previous methods. Anita McElroy has now successfully used this approach with the condors. Remarkably, she has also been successful at gender identification by amplifying the DNA present in bird droppings.

After years of working with condor gender identification, it is becoming clear that DNA may be present in various types of samples that were, up to now, considered unlikely sources of genetic information. Mr. Bo Ding, a visiting scientist from the Kunming Institute of Zoology in China, is studying giant panda genetics. He has shown that DNA sequences may be amplified by the PCR method from scent gland secretions left by giant pandas as they mark their territory. Mr. Ding has also been able to amplify DNA sequences from giant panda fecal samples, including those aged under field conditions for as long as two weeks. The ability to monitor and genetically identify giant panda populations without having to follow, capture, or anesthetize the animals provides an important new tool for conservation of wild populations. It will also help in the design and management of wildlife reserves.

As populations of free-ranging black rhinos increase in sanctuaries, scientists need the ability to collect and analyze genetic information from noninvasive samples. These could help to monitor these wild rhino populations. Mr. Ding has also been able to demonstrate that relatively small samples of rhino dung may serve as sources of DNA for amplification and analysis.

This concept that samples such as feathers, hair, saliva, scent marks, and fecal matter could be usefully exploited to provide genetic information was considered impossible before PCR was developed as a tool. The applications of noninvasive DNA analysis methods to conservation monitoring, assessment, and management are only in their early stages of development. However, they offer the potential for providing information that is crucial to evolving approaches to wildlife management for such threatened species as the California condor, giant panda, gorilla, and rhinoceros.

—Oliver Ryder, Ph.D.
Head of Genetics Division/CRES

Reproductive Physiology

Cells collected from the vagina of a female mammal convey important information about the animal's estrous cycle. Because the vagina is a "target organ" for the sex hormone estrogen, changes in the circulating levels of this hormone in the bloodstream are reflected in the appearance of vaginal cells. The simple, noninvasive swabbing technique is routinely performed on domestic cats and dogs, and it has been done at CRES on a wide variety of species including the elephant, koala, cuscus, clouded leopard, margay, and cheetah.

Cells are gently rolled on a microscope slide, fixed, and then stained with Papanicolaou (usually called PAP, the stain used in cancer screening of human female cervical cells). At least 100 cells from each slide are evaluated microscopically and classified by presence or absence of a nucleus, the nucleus:cytoplasm ratio, and the size, thickness, color, and shape of the cytoplasm. When a female has low circulating estrogen levels, her vaginal cells are predominantly small, round, and blue with a large nucleus. As estrogen levels rise with approaching estrus, the cells enlarge, become angular, stain pink, and have a small nucleus. When the female is in estrus, the high estrogen concentration transforms the vaginal cells into characteristic enucleated, orange cells called "keratinized" or "cornified."

The reproductive physiology division is currently evaluating swabs from Bai Yun, the female giant panda resident at the San Diego Zoo, to monitor her estrous cycle. The data generated will be combined with behavioral changes and urinary hormone levels to pinpoint the time of peak estrus and ovulation. Because a female panda is in estrus only a few days each year, these data could increase Bai Yun's chances of pregnancy, indicating to animal care staff when she should be paired with Shi Shi.

Comparative Physiology

The Lesser Antilles iguana is among the largest native land vertebrates of the Lesser Antilles islands in the Caribbean Sea. Widespread destruction of habitat and introduction of nonnative animals have contributed to the demise of this animal, as have the effects of hybridization with green iguanas and hunting by native peoples. Evidence of this unfortunate fact is found in its scientific name, *Iguana delicatissima*, meaning the "delicious iguana."

In 1994, the CRES comparative physiology division received a pair of Lesser Antilles iguanas, in the hope of developing a protocol for the successful captive propagation of this unique species of lizard. Only eight individuals of this species exist outside of the Lesser Antilles today, and they have never successfully reproduced in captivity. Last year, courtship and mating occurred among the iguanas at CRES, and at the Memphis Zoo and the Jersey Wildlife Preservation Trust, but none of the eggs that were laid hatched. Infertility and lower hatching success are common in the first year of reproduction for many iguanid species. Since the pair of iguanas at CRES were juveniles when they arrived, it is not surprising that their first clutch was infertile. Our hopes are high that the 1997 breeding season may produce the first offspring of this species to be propagated in captivity. If fertile eggs are laid, we plan to carry out an egg-incubation study to determine the optimal conditions for artificially hatching eggs of this endangered and little-known lizard.

Jeff Lemm

David Western: Innovative Leadership in Kenya

1997 Zoological Society of San Diego Conservation Medalist

THE ZOOLOGICAL SOCIETY OF SAN DIEGO
FOR CONSERVATION

Since 1966, the Zoological Society of San Diego has awarded its conservation medal to more than 40 distinguished scientists. For 1997, the recipient of the award is Dr. David Western, director of the Kenya Wildlife Service. Here Dr. Western (holding microphone) discusses exhibits with Kenya's President Daniel arap Moi during the KWS 50th anniversary celebrations in August 1996.

> ***"I am also interested in the broader theme of environment, conservation and development at a regional and global level."—David Western***

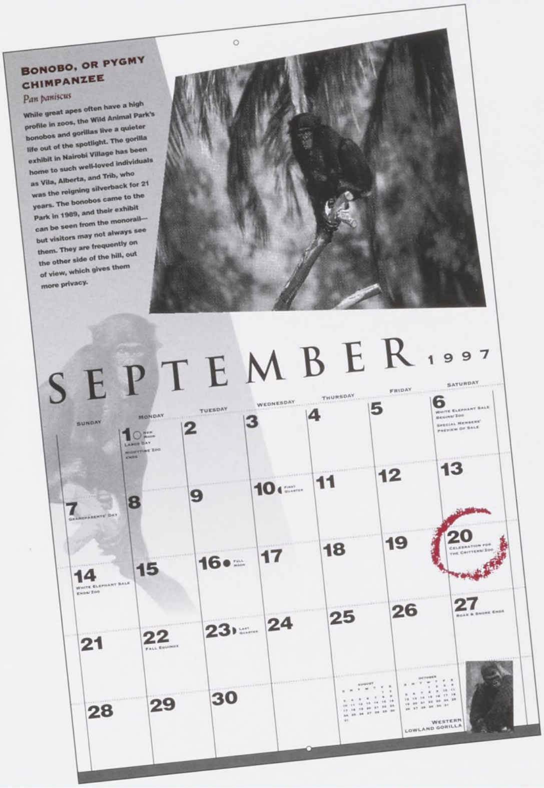
Amboseli, one of Kenya's magnificent national parks, has been the setting for the past 28 years of Dr. David Western's work. A native Kenyan, he observes that "My primary interest is the conservation of African savannas. I approach this from a broad perspective, from both a human and ecological angle. From the human angle, I am interested in making conservation acceptable and effective by involving a wide range of participants, most especially local landowners. I am also interested in the broader theme of environment, conservation and development at a regional and global level."

A long-term approach in the application of scientific solutions to problems in conservation is Dr. Western's hallmark. His study over the years of a savanna ecosystem led to the application of ecological knowledge to the management of Amboseli. "Theoretically, wildlife interactions with livestock can tell us a great deal about competition and new ways to manage and conserve ecosystems." With an added focus on community awareness—moving from centralized to locally based programs and an integrated approach to conservation and development—he has brought Kenya to the attention of other African nations and the international community. This work led directly to Kenya's new wildlife policies, which began in the mid-1970s. With the aim of integrating tourism and conservation planning—making tourism into a positive force for conservation—it has become the model for other parks in Africa.

The 1997 Conservation Medal is awarded to Dr. Western in recognition of the impact he has had on an entire generation of scientists and students in Kenya. His approach looks for ways to engage and train African students and scholars, and he established the African Conservation Centre in Nairobi, which offers a facility devoted to the development of excellence in conservation studies. Beyond savannas, the Centre tackles issues confronting other African habitats, from coral reefs to tropical forests. By encouraging local communities to link tourism and development with conservation strategies, this has led to many parallel research projects beneficial to wildlife conservation in other parts of Africa. His leadership in programs to conserve Kenya's endangered black rhinoceros, as well as elephants, also demonstrates that dedication and innovative leadership can conserve wildlife resources and benefit local communities.

By popularizing and interpreting conservation locally and internationally over three decades—through television documentaries, news interviews, books, and articles—Dr. Western has proven to be a longtime believer in grass roots conservation education. As the present director of Kenya Wildlife Service, chairman of the Wildlife Clubs of Kenya, member of the board of directors for the Kenya Tourism Board, and adjunct professor with the University of California, San Diego, he continues his extraordinary life's work in the cause of conservation.

Save the Date for Wild About Zoo!



BONOBO, OR PYGMY CHIMPANZEE

Pan paniscus

While great apes often have a high profile in zoos, the Wild Animal Park's bonobos and gorillas live a quieter life out of the spotlight. The gorilla exhibit in Nairobi Village has been home to such well-loved individuals as Vila, Alberta, and Trib, who was the reigning silverback for 21 years. The bonobos came to the Park in 1989, and their exhibit can be seen from the monorail—but visitors may not always see them. They are frequently on the other side of the hill, out of view, which gives them more privacy.

SEPTEMBER 1997

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
	1 ○ New Moon Labor Day Nighttime Zoo Ends	2	3	4	5	6 White Elephant Sale Begins/Zoo Special Members' Preview of Sale
7 Grandparents' Day	8	9	10 ◐ First Quarter	11	12	13
14 White Elephant Sale Ends/Zoo	15	16 ● Full Moon	17	18	19	20 Celebration for the Critters/Zoo
21	22 Fall Equinox	23 ◑ Last Quarter	24	25	26	27 Roar & Snore Ends
28	29	30		August	October Western Lowland Gorilla	

Party under the stars to help save the world's wildlife! Mark your calendars now for the San Diego Zoo's annual Celebration for the Critters, Wild About Zoo!, on Saturday, September 20, from 7 P.M. to midnight. A special VIP festivity will begin at 6:15 P.M. This beastly bash in the wilds of the Zoo benefits CRES, the Zoological Society of San Diego's Center for Reproduction of Endangered Species, and its global conservation efforts. Last year's event broke all records, with more than $100,000 raised for CRES.

Highlights of this roaring good time include an abundance of exotic "taste treats" from many of San Diego's finest restaurants and beverage vendors. Leap, hop, and prance to a variety of entertainment, featuring the rock-and-roll beats of Rockola. The ever popular Silent Auction will offer a vast array of fabulous gifts, such as dining and weekend getaway packages. And to add a few monkeyshines to your evening, try your skill at the Arcade's rib-tickling games. Above all the fun, the Skyfari will remain open for the evening for panoramic vistas of the Zoo and the twinkling lights of the Celebration.

Tickets are $40 per person ($50 after September 12) and will go on sale this summer. Guests must be 21 years of age or older to attend. Be Wild About Zoo! by adding your name to the mailing list. Just call (619) 557-3974 between the hours of 8 A.M. and 4 P.M.

There's Added Value to Matching Gifts

Did you know that your support of the Zoological Society of San Diego can grow through participation in our Matching Gifts Program? Matching gifts can make optimum use of the resources available to you through your employer that, in turn, can increase financial contributions to the Center for Reproduction of Endangered Species (CRES). The leadership provided by those individuals who match their own contributions through this program is important to the continued success of our conservation programs at CRES. Employees—and often retirees—are eligible for matching gifts, and many companies in the San Diego community participate in this type of charitable giving. To find out more about the giving opportunities that are available through matching gifts, contact your employer or Laurie Chadwick in the Zoological Society's development office at (619) 685-3219.

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You Can Endow the Endowment!

Created in 1980, the CRES Endowment is a special restricted fund established by the Zoological Society of San Diego to provide annual income to help support vital CRES activities. Contributions are added to the fund principal, which remains invested in perpetuity: only the annual income is used. Accordingly, your gift to the Endowment will go on providing funds for CRES programs for many years to come.

There are many tax-advantaged ways to contribute:

- Cash, which is completely tax deductible.
- Gifts of stock are especially advantageous because you can deduct the full market value, even if highly appreciated.
- You can become a participant in the Life Income Fund, receive tax deductions, a lifetime income stream, and ultimately make a significant contribution to the CRES Endowment.
- Gifts can also be made via your will, trust, life insurance, or an IRA account.

To discuss any of these possibilities, please call Patrick O'Reiley, the Zoological Society's estate gifts director, on his private line at (619) 685-3215.

CRES REPORT
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SAN DIEGO ZOO · SAN DIEGO WILD ANIMAL PARK

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